

**What is claimed is:**

1. Fermentation process for the preparation of L-amino acids, especially L-threonine, wherein the following steps are carried out:
  - 5        a) fermentation of the microorganisms of the family Enterobacteriaceae producing the desired L-amino acid, in which microorganisms at least the pckA gene or nucleotide sequences coding therefor are attenuated and, in particular, switched off,
  - 10      b) enrichment of the L-amino acid in the medium or in the bacterial cells, and
  - 15      c) isolation of the L-amino acid, constituents of the fermentation broth and the biomass in its entirety or portions thereof optionally being isolated as a solid product together with the L-amino acid.
2. Process according to claim 1, wherein microorganisms are used in which other genes of the biosynthetic pathway of the desired L-amino acid are additionally amplified.
- 20     3. Process according to claim 1, wherein microorganisms are used in which the metabolic pathways which reduce the formation of the desired L-amino acid are at least partially switched off.
- 25     4. Process according to claim 1, wherein the expression of the polynucleotide(s) coding for the pckA gene is attenuated and, in particular, switched off.
- 30     5. Process according to claim 1, wherein the regulatory and/or catalytic properties of the polypeptide (enzyme protein) coded for by the polynucleotide pckA are reduced.

6. Process according to claim 1, wherein microorganisms of the family Enterobacteriaceae in which one or more genes selected from the group comprising:

- 5        6.1      the thrABC operon coding for aspartate kinase, homoserine dehydrogenase, homoserine kinase and threonine synthase,
- 10      6.2      the pyc gene coding for pyruvate carboxylase,
- 15      6.3      the pps gene coding for phosphoenolpyruvate synthase,
- 20      6.4      the ppc gene coding for phosphoenolpyruvate carboxylase,
- 25      6.5      the pntA and pntB genes coding for transhydrogenase,
- 30      6.6      the rhtB gene for homoserine resistance, and
- 35      6.7      the rhtC gene for threonine resistance,
- 40      6.8      the gdhA gene coding for glutamate dehydrogenase

are simultaneously amplified and, in particular, overexpressed are fermented for the preparation of L-amino acids.

20      7. Process according to claim 1, wherein microorganisms of the family Enterobacteriaceae in which one or more genes selected from the group comprising:

- 25      7.1      the tdh gene coding for threonine dehydrogenase,
- 30      7.2      the mdh gene coding for malate dehydrogenase,

- 7.3 the gene product of the open reading frame (orf) yjfA, and
- 7.4 the gene product of the open reading frame (orf) ytfP,
- 5 are attenuated and, in particular, switched off, or the expression is reduced, are fermented for the preparation of L-amino acids.
8. Fermentation process for the preparation of L-amino acids, especially L-threonine, wherein the following steps are carried out:
- 10 a) fermentation of the microorganisms of the family Enterobacteriaceae producing the desired L-amino acid, in which microorganisms at least the open reading frames yjfA and/or ytfP or nucleotide sequences coding therefor are attenuated and, in particular, switched off,
- 15 b) enrichment of the L-amino acid in the medium or in the bacterial cells, and
- 20 c) isolation of the L-amino acid, constituents of the fermentation broth and the biomass in its entirety or portions thereof optionally being isolated as a solid product together with the L-amino acid.
- 25 9. Process according to claim 1 or 8, wherein L-isoleucine, L-valine, L-lysine or L-threonine is prepared.
10. L-Amino acid-producing microorganisms of the family Enterobacteriaceae in which at least the pckA gene or nucleotide sequences coding therefor are attenuated and, in particular, switched off.

11. L-Amino acid-producing microorganisms of the family Enterobacteriaceae according to claim 10, which additionally have one or more features selected from the group comprising: a resistance to  $\alpha$ -amino- $\beta$ -hydroxyvaleric acid, an amplified homoserine dehydrogenase I-aspartate kinase I in the feed back resistant form, an optionally compensable partial need for L-isoleucine, an attenuated threonine dehydrogenase and the ability to utilize sucrose.
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- 10 12. L-Amino acid-producing microorganisms of the family Enterobacteriaceae, in which at least the open reading frame yjfA and/or ytfP or nucleotide sequences coding therefor are attenuated and, in particular, switched off.
- 15 13. L-Amino acid-producing microorganisms of the family Enterobacteriaceae according to claim 12, which additionally have one or more features selected from the group comprising: a resistance to  $\alpha$ -amino- $\beta$ -hydroxyvaleric acid, an amplified homoserine dehydrogenase I-aspartate kinase I in the feed back resistant form, an optionally compensable partial need for L-isoleucine, an attenuated threonine dehydrogenase and the ability to utilize sucrose.
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- 25 14. Plasmid pMAK705 $\Delta$ pckA, shown in Figure 1, containing parts of the 5' and 3' regions of the pckA gene, corresponding to SEQ ID No. 3.
- 30 15. Plasmid pMAK705 $\Delta$ yjfA, shown in Figure 2, containing the 5' and 3' flanks of the ytfP-yjfA region, including very short residues of the open reading frames yjfA- and ytfP, corresponding to SEQ ID No. 6.
16. Plasmid pMAK705 $\Delta$ 90bp, shown in Figure 5, containing the 5' and 3' flanks of the ytfP-yjfA region, including very

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- short residues of the open reading frames yjfA- and ytfP, corresponding to SEQ ID No. 7.
17. Isolated polynucleotide from microorganisms of the family Enterobacteriaceae containing a polynucleotide sequence coding for the 5' and 3' regions of the pckA gene, shown in SEQ ID No. 4, which is particularly suitable as a constituent of plasmids for the position-specific mutagenesis of the pckA gene.
18. Isolated polynucleotide from microorganisms of the family Enterobacteriaceae containing the 5' and 3' flanks of the ytfP-yjfA region, shown in SEQ ID No. 6, which is particularly suitable as a constituent of plasmids for the position-specific mutagenesis of the open reading frames ytfP and/or yjfA.
19. L-Threonine-producing strains of the family Enterobacteriaceae containing a deletion mutation in the pckA gene, corresponding to SEQ ID No. 4.
20. L-Threonine-producing strains of the family Enterobacteriaceae containing a deletion mutation in the open reading frame ytfP, corresponding to SEQ ID No. 6 or 7.
21. L-Threonine-producing strains of the family Enterobacteriaceae containing a deletion mutation in the open reading frame yjfA, corresponding to SEQ ID No. 6 or 7.
22. L-Threonine-producing strains of the family Enterobacteriaceae according to claim 19, additionally containing a deletion mutation in the open reading frame ytfP, corresponding to SEQ ID No. 6 or 7.
23. L-Threonine-producing strains of the family Enterobacteriaceae according to claim 19, additionally

containing a deletion mutation in the open reading frame yjfA, corresponding to SEQ ID No. 6 or 7.

24. L-Threonine-producing strains of the family Enterobacteriaceae according to claims 19, 20 or 21,  
5 wherein they have one or more features selected from the group comprising: a resistance to  $\alpha$ -amino- $\beta$ -hydroxyvaleric acid, an amplified homoserine dehydrogenase I-aspartate kinase I in the feed back resistant form, an optionally compensable partial need  
10 for L-isoleucine, an attenuated threonine dehydrogenase and the ability to utilize sucrose.
25. Escherichia coli K-12 strain MG442 $\Delta$ pckA deposited under number DSM 13761 at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures).  
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26. Escherichia coli K-12 strain MG442 $\Delta$ 90yjfA deposited under number DSM 14289 at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures).  
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27. Escherichia coli K-12 strain B3996kur $\Delta$ tdhpckA/PVIC40, deposited under number DSM 14150 at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures).